Dorsal medullary 5-HT_3 receptors and sympathetic premotor neurones in the rat

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1. Our aim was to determine whether the cardiovascular neurones in the rostro-ventrolateral medulla (CV-RVLM neurones) were involved in the sympathoexcitation induced by stimulation of 5-HT_3 receptors in the region of the nucleus tractus solitarii (NTS). Experiments were performed in pentobarbitone-anaesthetized rats, artificially ventilated and paralysed with pancuronium bromide.

2. Using extracellular recordings, different types of RVLM neurones were characterized: cardiovascular (CV), ventilation-related and baroreflex-insensitive (unidentified) neurones. The CV-RVLM cells were further subdivided into three populations according to their axonal conduction velocities: A ($1{\cdot}2 \pm 0{\cdot}1$ m s^{-1}), B ($2{\cdot}5 \pm 0{\cdot}2$ m s^{-1}) and C ($6{\cdot}8 \pm 1{\cdot}1$ m s^{-1}).

3. Only the CV-RVLM neurones of the A and B categories were partially inhibited (−30 %) by a hypotensive dose ($2{\cdot}5$ μg kg^{-1} I.V.) of clonidine.

4. Microinjections into the region of the commissural NTS of 1-(*m*-chlorophenyl)-biguanide (CPBG, 2 nmol), a selective 5-HT_3 receptor agonist, elicited an increase in both lumbar sympathetic nerve discharge (SND) and arterial pressure. In addition, this treatment produced a marked excitation of CV-RVLM neurones of the A and B categories, without affecting those of the C type, as well as ventilation-related and unidentified RVLM cells.

5. The activity of the CV neurones in the caudo-ventrolateral part of the medulla oblongata (CV-CVLM) was not modified by 5-HT_3 receptor stimulation in the NTS.

6. Prior intra-NTS microinjections of ondansetron (300 pmol, a selective 5-HT_3 receptor antagonist) into the region of the commissural NTS prevented the excitation of A and B CV-RVLM neurones induced by CPBG.

7. Intracarotid administration of saline saturated with CO_2 (chemoreceptor activation) elicited both an increase in the SND and an excitation of the clonidine-insensitive CV-RVLM neurones of the C type, without affecting A and B neurones.

8. In conclusion, the sympathoexcitation elicited following 5-HT_3 receptor stimulation in the region of the commissural NTS of pentobarbitone-anaesthetized rats seems to result from the excitation of two different pools of clonidine-sensitive CV-RVLM neurones. These neurones are apparently not involved in the sympathetic component of the chemoreceptor reflex.

The rostro-ventrolateral medulla (RVLM) and nucleus tractus solitarii (NTS) are critically involved in the reflex control of sympathetic activity (Guyenet, Filtz & Donaldson, 1987; Sun & Guyenet, 1987; Spyer, 1994).

The RVLM contains neurones that receive a number of inputs both peripheral and central in origin that influence sympathetic nerve activity (Sun & Guyenet, 1987; Spyer, 1994). Previous reports have described two populations of cardiovascular (CV)-RVLM neurones that project to the thoracic spinal cord (Brown & Guyenet, 1985; Sun & Guyenet, 1985). The first population consists of clonidine-sensitive cells with slow-conducting axons. The second population corresponds to cells that do not respond to hypotensive doses of clonidine, and are characterized by a much higher conduction velocity (Sun & Guyenet, 1986).

The NTS is the site of termination of afferent fibres arising from arterial baroreceptors (baroreflex), cardiopulmonary chemoreceptors (Bezold–Jarisch reflex) and carotid chemoreceptors (chemoreflex) (Palkovits & Zaborsky, 1977; Kalia & Mesulam, 1980; Jordan & Spyer, 1986). For baro- and Bezold–Jarisch reflexes, which seem to share the same integrating mechanisms and pathways in the brain (Verberne & Guyenet, 1992), second-order neurones project from the NTS to the caudal ventrolateral part of the medulla (CVLM)

(Gordon 1987; Guyenet *et al.* 1987; Verberne & Guyenet, 1992). These neurones exert an excitatory action on the GABAergic CVLM neurones that project to the RVLM where they inhibit the CV-RVLM neurones of this pressor area (Brown & Guyenet, 1985; Sun & Guyenet, 1985; Jeske, Reis & Milner, 1995). Some of the CV-RVLM neurones additionally constitute an efferent link in the sympathetic component of the chemoreflex (Guyenet & Brown, 1986; Sun & Reis, 1995). Indeed, it has been demonstrated that some NTS chemosensitive neurones have axonal projections to the RVLM (Koshiya & Guyenet, 1996).

Within the NTS, serotonin (5-hydroxytryptamine, 5-HT) seems to be involved in the reflex control of blood pressure. Studies in both anaesthetized and conscious rats have shown that 5-HT_2 receptor stimulation in the NTS elicits the typical CV responses of baroreceptor activation (Merahi, Orer & Laguzzi, 1992*a*; Callera, Bonagamba, Sévoz, Laguzzi & Machado, 1997*a*). On the other hand, stimulation of NTS 5-HT_3 receptors elicits a chemoreceptor-like increase in arterial pressure and lumbar sympathetic activity (Merahi, Orer, Laporte, Gozlan, Hamon & Laguzzi, 1992*b*; Callera, Sévoz, Laguzzi & Machado, 1997*b*). In experiments aimed at analysing the mechanism(s) responsible for this sympatho-excitatory effect, we observed that stimulation of 5-HT_3 receptors in the NTS did not inhibit the sympathetic component of the baroreflex (Nosjean, Franc & Laguzzi, 1995). Accordingly, it can be inferred that 5-HT_3 receptor-mediated sympathoexcitation is not the consequence of the disruption of tonic baroreceptor sympathoinhibitory messages. However, our finding is compatible with the idea that CV-RVLM neurones may be involved in the sympathoexcitatory effect of 5-HT_3 receptor stimulation in the NTS. In other experiments, we also observed that prior microinjections of pressor (nanomolar) doses of 5-HT_3 receptor agonists into the NTS did not increase the sympathetic chemoreflex response (Sévoz, Callera, Machado, Hamon & Laguzzi, 1997). However, this observation does not rule out the possibility that under some physiological conditions, 5-HT released in the NTS may excite the NTS–RVLM sympathetic chemoreflex pathway (Koshiya & Guyenet, 1996), and the CV-RVLM neurones involved in this reflex. Indeed, as previously observed with the pressor chemoreflex response (Sun & Reis, 1995), we recently found that the microinjection of kynurenic acid, a glutamate receptor antagonist, into the RVLM blocked the pressor effects elicited by 5-HT_3 receptor stimulation in this area (Sévoz, Hamon & Laguzzi, 1996*b*).

In order to elucidate the possible role of CV-RVLM neurones in the sympathetic response to 5-HT_3 receptor stimulation in the NTS, we have analysed the effects of intra-NTS microinjections of a potent and selective 5-HT_3 receptor agonist, 1-(*m*-chlorophenyl)-biguanide (CPBG), as well as the effects of chemoreflex activation, on the activity of the different categories of RVLM neurones. In addition, the possible effects of intra-NTS administration of CPBG on the CV-CVLM neurones were also investigated.

METHODS

General procedures

Experiments were performed on 133 adult male Sprague–Dawley rats (body weight, 290–320 g) under sodium pentobarbitone anaesthesia (60 mg kg^{-1} I.P.). Procedures involving animals and their care were all conducted to conform with the institutional guidelines, which are in compliance with national and international law and policies (council directive no. 87-848, 19 October 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions no. 0299 to M.H. and no. 0314 to R.L.)

A cannula was inserted into the left femoral vein for injections of drugs and additional doses of sodium pentobarbitone if required (see below). Mean arterial pressure (MAP) was monitored through a catheter inserted into the left common carotid artery.

After location of the retrofacial region of the RVLM (see below, 'RVLM single-unit recordings'), rats were paralysed with pancuronium bromide (1 mg kg^{-1} I.V., a dose sufficient to paralyse the animal until the end of the experiment) and ventilated with room air through a tracheal cannula, connected to an artificial ventilator. End-tidal CO_2 was maintained close to 4% by adjusting the ventilation rate and/or the volume. Before paralysis, the depth of anaesthesia was assessed by pinching the hindpaw. In case of withdrawal reflex, a supplementary dose of sodium pentobarbitone was given (5–10 mg kg^{-1} I.V.). After paralysis, whenever small changes in MAP and/or heart rate (HR) were observed in response to pinching of the hindpaw, an additional dose of anaesthetic was given (5–10 mg kg^{-1} sodium pentobarbitone I.V.) in order to eliminate the cardiovascular responses. Core body temperature was monitored and maintained at 37 °C with a heating pad. At the end of the experiments, rats were killed by an overdose of sodium pentobarbitone and their brains were removed for histological controls.

Microinjections into the NTS

Rats were placed in a Kopf stereotaxic frame with the head fixed horizontally. The dorsal surface of the brainstem was exposed through a limited occipital craniotomy. Single-barrel glass micropipettes (70–90 μm external diameter), connected to a Hamilton microsyringe filled with solutions of predetermined doses of 5-HT_3 receptor ligands (see Sévoz, Nosjean, Callera, Machado, Hamon & Laguzzi, 1996*b*), or saline, were lowered bilaterally into the commissural NTS at the level of the calamus scriptorius, at similar sites to those selected in a previous study (Sévoz *et al.* 1996*b*). Microinjections (0·1 μl) were made over 1 s using a pneumatic microinfusion pump. In experiments aimed at studying 5-HT_3 receptor agonist–antagonist interactions, prior microinjections were made with the antagonist; 2 min later, another micropipette filled with the agonist was lowered for microinjections into the same sites in the NTS. Repeated microinjections into the same sites were possible because the hole produced in the NTS by the first microinjections was always clearly visible using a microscope. Moreover, experiments that were taken into account in the present study were those for which the introduction of the micropipette into the brainstem during repeated injections did not produce any tissue depression.

Recording of the sympathetic nerve activity

The procedures used for recording and processing the lumbar sympathetic nerve discharge (SND) have been described in detail elsewhere (Verberne & Guyenet, 1992; Nosjean *et al.* 1995; Sévoz *et al.* 1997). Briefly, the lumbar sympathetic nerve was isolated between

L3 and L5, placed uncut on bipolar silver hook electrodes and surrounded with Sil-Gel (Rhodosil, Rhône-Poulenc-Rorer, Vitry, France). The recorded SND was amplified and filtered (100–3000 Hz, 50 Hz notch filter), and the resulting signal was rectified and subjected to analog integration (Gould, 13-G4615-70) with a resetting time of 1 s. Then SND was digitized and stored on a digital tape recorder (DRT 1800, Biologic) along with cardiovascular variables. The residual electrical noise remaining after a large dose of clonidine (200 μg kg^{-1}, I.V.) was taken as the zero level of SND (Verberne & Guyenet, 1992). SND was expressed in arbitrary units, zero being the electrical noise (after clonidine) and 100 units corresponding to the resting level measured during the control period. MAP and heart rate signals were also digitized (sampling rate, 0·1 s) and averaged during consecutive 1 s intervals. Data processing was performed using a computer program developed in our laboratory.

RVLM single-unit recordings

A hole 5 mm in diameter, with the centre 2 mm lateral (right side) to the interparietal suture bone and 3 mm rostral to the calamus scriptorius, was drilled through the skull. A bipolar concentric stimulating electrode (Phymep, SNEX 100) was placed in the fascia surrounding the mandibular branch of the facial nerve in order to locate the motor facial nucleus by means of antidromic field potential recordings (5 mA, 200 μs, 1·5 Hz; Brown & Guyenet, 1985). The RVLM and the CVLM neurones were recorded just ventrally and 0–500 μm or 1·5–1·8 mm posterior to the caudal end of the facial motor nucleus (retrofacial region of the RVLM), respectively.

Single-unit recordings were obtained using microelectrodes (tip diameter, 3·5 μm) made of borosilicate glass (GC 150F-10, Clark Electromedical) filled with 2 M NaCl alone or 2 M NaCl containing 1% Fast Green. Impedances of these electrodes were usually 5–12 MΩ. Signals were filtered (100–3000 Hz, 50 Hz notch filter), monitored on an oscilloscope and recorded on a tape recorder (DTR 1800). The unit discharges, digitized by a window discriminator, were counted during intervals of 1 s and recorded as an integrated activity histogram.

Electrical stimulation of the spinal cord

After a thoracic laminectomy (T_2–T_3), a second bipolar stimulating electrode was implanted on the right side of the spinal cord, in the thoracic region, with its tip 1·0 mm below the dorsolateral sulcus for performing the test of antidromic activation of the recorded CV-RVLM neurones (Sun & Guyenet, 1986). Stimulation parameters were 2 mA, 200 μs and 1 Hz, until a reticulospinal unit was detected by an evoked action potential. To verify the antidromicity of this activation, frequency was increased up to 50 Hz. Under such conditions, antidromic potentials were evoked exactly with the same latency after the stimulation of the spinal cord. Stimulation intensity was then set to just above the threshold level (0·5 mA), and frequency was reduced to 1 Hz, for the collision test between the evoked and the spontaneous potential (Sun & Guyenet, 1986). When the basal discharge rate of the recorded cell was initially of $\geqslant$ 10 spikes s^{-1} (for a baseline MAP of 90 mmHg), it was then reduced by aortic occlusion in order to avoid systematic collision between the spontaneous and the evoked potentials. In addition, the spontaneous unit discharge, through the window discriminator, was used to trigger the oscilloscope and to deliver the spinal stimulus at a variable delay. Of a total of 110 CV-RVLM cells, eighty were found to project to the spinal cord.

Baroreceptor reflex activation

An inflatable cuff (snare) made of heat-stretched soft plastic tubing was wrapped around the descending aorta below the diaphragm, and its external part connected to a 1 ml syringe (Sun & Guyenet, 1986). Thus the snare could gradually constrict the aorta (aortic occlusion), transiently elevating MAP (~40 mmHg from a baseline range of 85–100 mmHg) and exciting, by non-pharmacological means, arterial baroreceptors as well as other peripheral receptors such as cardiopulmonary and renal baroreceptors. Plots of unit activity (spikes s^{-1}) *vs.* MAP (baroreceptor curves) were generated by the software. As previously indicated (Brown & Guyenet, 1985), these plots consist of a plateau below baroreceptor threshold (maximum unit discharge) followed by a linearly decrementing phase intersecting the *x*-axis at a MAP defined as the 'cut-off MAP'. The maximum unit activity was usually measured as the peak discharge occurring during the brief hypotensive period ($40 < \Delta MAP < 50$ mmHg) consecutive to the relaxation of the aortic snare.

In some experiments, baroreceptor activation was also induced by an intravenous bolus of phenylephrine at a dose (5–10 μg kg^{-1}) sufficient to elevate the MAP by 40 mmHg (from a baseline range of 85–100 mmHg).

Bezold–Jarisch reflex activation

Phenylbiguanide was injected into the right atrium through a cannula inserted into the left jugular vein at a dose (40 μg kg^{-1} in 0·1 ml) sufficient to stimulate the cardiopulmonary receptors (Vardhan, Kachroo & Sapru, 1993*a*).

Chemoreflex activation

Chemoreceptor reflex responses, tachypnoea and increases in MAP, were evoked by the I.V. administration (0·3 ml in 8 s) of saline saturated with 100% CO_2, through a cannula inserted into the right external carotid artery (with its tip at the origin of the small artery supplying the carotid body). In some rats, bilateral chemodenervation was also performed by resection of the carotid body and its small supplying artery. This procedure is well known to prevent completely the respiratory and sympathetic chemoreflex responses (Vardhan, Kachroo & Sapru, 1993*b*; Sévoz *et al.* 1997).

Histology

Methylene Blue (0·1 μl in 1 s) was microinjected at the end of most of the experiments for the histological control of the injection sites in the NTS. The dye spread over ~0·5 mm from the injection point.

Recording sites in the RVLM were always labelled by the iontophoretic ejection of 1% Fast Green (20 μA negative DC current, 20 min). Rats were then perfused intracardially with saline and a solution of 4% paraformaldehyde in 0·1 M sodium phosphate at pH 7·4. After perfusion, the brain was removed from the skull and coronal sections (60 μm) of the medulla oblongata were cut using a microtome and stained with Nissl substance.

Statistical analysis

After verification that the experimental values were normally distributed, Student's paired *t* test was used. Each animal was used as its own control (pretreatment period). A difference was considered to be significant at $P < 0{\cdot}05$. All values are expressed as means ± standard error of the mean (S.E.M.).

Drugs

Drugs used were CPBG (Research Biochemical Incorporation, Natick, MA, USA), ondansetron (Glaxo, Ware, UK), phenylbiguanide (Aldrich, Strasbourg, France), clonidine and pancuronium bromide (Sigma). All drugs were dissolved in saline.

RESULTS

Identification of neurones in the vasomotor centre of the RVLM

Cardiovascular neurones

A total of eighty CV-RVLM neurones, in seventy rats, which satisfied the following criteria, were recorded: barosensitivity, averaged discharge synchronized with the pulse and axonal projection to the spinal cord (Brown & Guyenet, 1985; Guyenet & Brown, 1986). The neurones that were barosensitive but did not fill the other two criteria (25% of the recorded cells) were not considered in our study. The CV-RVLM neurones were found over 400 μm in a region just caudal to the end of the facial nucleus (see Methods) (2·5–2·9 mm rostral to the calamus scriptorius), within 400–600 μm from the ventral medullary surface and 1·8–1·9 mm lateral to the midline (Fig. 1*Aa*).

According to the criteria given above, CV-RVLM neurones were identified by a rapidly developing decrease in their firing rate during a rise in arterial pressure (baroreceptor activation) produced by a gradual (5 s) aortic occlusion or a bolus administration of phenylephrine (5–10 μg kg^{-1} I.V.) (Fig. 2*A* and *Ba*). In addition, these neurones were pulse synchronized when MAP was $\geqslant$ 100 mmHg (Fig. 2*Ca*).

These CV-RVLM neurones could be activated by antidromic stimulation (see Methods) of the intermediate lateral column of the thoracic spinal cord (Fig. 3). The antidromic latencies of these neurones varied from 4 to 32 ms. Their axonal conduction velocities, calculated using these latencies and the distance between the spinal stimulatory electrode and the RVLM recording electrode (30 mm), were in the range of 0·9–7·5 m s^{-1}, similar to the conduction velocity profiles already reported for these neurones (Sun & Guyenet, 1986).

Under our experimental conditions, consideration of the conduction velocities, the basal firing rate and the sensitivity to baroreceptor activation allowed the distinction of three different populations of CV-RVLM neurones.

The first pool of these neurones (group A, $n = 20$) was characterized by the slowest conduction velocity (1·2 $\pm$ 0·1 m s^{-1}), ranging between 0·9 and 1·8 m s^{-1} (Fig. 3*A*) and the lowest firing rate (6·5 $\pm$ 0·2 spikes s^{-1}) when recorded at MAP $\leqslant$ 70 mmHg (plateau of the baroreceptor curve, see Methods and Fig. 4). For this pool of neurones, the slope of the baroreceptor curve was equal to 0·13 $\pm$ 0·01 spikes s^{-1} mmHg^{-1}. This slope was defined as the gain of the sympathetic baroreflex (Nosjean *et al.* 1995). In addition, its cut-off MAP (see Methods) was 125·0 $\pm$ 3·2 mmHg (Fig. 4).

The second pool of CV-RVLM neurones (group B, $n = 30$) had intermediate conduction velocity (2·5 $\pm$ 0·2 m s^{-1}, range 2·1–3·0 m s^{-1}, Fig. 3*B*) and firing rate (14·0 $\pm$ 0·5 spikes s^{-1}) when recorded at MAP $\leqslant$ 70 mmHg (Fig. 4). The slope of their baroreceptor curve was equal to 0·20 $\pm$ 0·01 spikes s^{-1} mmHg^{-1} and the cut-off MAP was 140·5 $\pm$ 3·5 mmHg (Fig. 4).

Finally, the third pool of CV-RVLM neurones (group C, $n = 30$) presented the fastest conduction velocity (6·8 $\pm$ 1·1 m s^{-1}, range 4·0–7·5 m s^{-1}, Fig. 3*C*) and the highest firing rate (23·0 $\pm$ 0·5 spikes s^{-1}) when recorded at MAP $\leqslant$ 80 mmHg (Fig. 4). The slope of their baroreceptor curve was equal to 0·35 $\pm$ 0·02 spikes s^{-1} mmHg^{-1} and the cut-off MAP was 145·0 $\pm$ 2·1 mmHg (Fig. 4).

Comparison of the baroreflex curves for the three distinct pools, A, B and C, of CV-RVLM neurones, showed that their slopes were significantly different ($P < 0·05$).

In agreement with previous reports (Sun & Guyenet, 1986), some of the CV-RVLM neurones were affected by the administration of hypotensive doses of clonidine, a mixed α_2 and imidazolic receptor agonist. At the dose of 2·5 μg kg^{-1}, clonidine ($n = 30$) produced a significant hypotension (ΔMAP, $-20·0 \pm 2·5$ mmHg from a resting MAP of 90·3 $\pm$ 5·2 mmHg, $P < 0·05$) and an inhibition of the discharge of neurones of the A (firing rate, 3·4 $\pm$ 0·2 spikes s^{-1} from a resting firing rate of 4·8 $\pm$ 0·1 spikes s^{-1}, $P < 0·05$, $n = 10$) and B (firing rate, 6·0 $\pm$ 0·5 spikes s^{-1} from a resting firing rate of 9·2 $\pm$ 0·4 spikes s^{-1}, $P < 0·05$, $n = 10$) categories, without affecting that of C neurones (17·5 $\pm$ 0·5 and 17·0 $\pm$ 0·3 spikes s^{-1}, before and after clonidine, respectively, $n = 10$).

Interestingly, all the CV-RVLM neurones were inhibited by activation of the Bezold–Jarisch reflex. Thus intra-atrial injection of phenylbiguanide (PBG, 40 μg kg^{-1}, $n = 30$) not only elicited the typical Bezold–Jarisch CV reflex responses: hypotension (ΔMAP, $-33·0 \pm 2·1$ mmHg from a resting MAP of 94·0 $\pm$ 3·5 mmHg, $P < 0·05$) and bradycardia (ΔHR, $-198·5 \pm 1·2$ beats min^{-1} from a baseline of 420·5 $\pm$ 5·0 beats min^{-1}, $P < 0·05$), but also completely inhibited the firing of all tested neurones in the three categories A, B and C ($n = 10$ for each category, Fig. 2*A*).

Ventilation-related neurones

At the same level or just dorsal (0–200 μm) to the CV-RVLM neurones, single-unit recordings (performed in fifteen rats) allowed the identification of neurones that discharged synchronously with inspiratory and expiratory movements generated by the ventilator. These neurones were called 'ventilation-related RVLM neurones' and seemed to be different, because of their ventral location, from the more dorsal respiratory neurones of the Bötzinger complex (Smith, Ellenberger, Ballanyi, Richter & Feldman, 1991). The latter cells were not recorded in our study. The discharge of the ventilation-related RVLM neurones was not significantly affected by the snare-evoked rise in arterial pressure (firing rate, 18·0 $\pm$ 2·5 spikes s^{-1} from a resting activity of 20·5 $\pm$ 2·2 spikes s^{-1}, $n = 7$, Fig. 2*Bb*) or the activation of the Bezold–Jarisch reflex (firing rate, 20·5 $\pm$ 5·2 spikes s^{-1} from a resting activity of 21·3 $\pm$ 4·5 spikes s^{-1}, $n = 8$). In addition, these ventilation-related neurones were not pulse synchronized (Fig. 2*Cb*).

Unidentified neurones

In the same retrofacial region where the CV-RVLM neurones were localized, we found (in fifteen rats) cells that did not respond to the snare-induced rise in MAP (firing rate, 10·5 ± 2·2 spikes s^{-1} from a resting activity of 11·3 ± 2·5 spikes s^{-1}, $n = 15$, Fig. 2*Bc*). In the same manner, activation of the Bezold–Jarisch reflex failed to affect the discharge of these cells (firing rate, 10·3 ± 1·5 spikes s^{-1} from a resting activity of 10·5 ± 2·5 spikes s^{-1}, $n = 5$). Furthermore, these cells were neither pulse synchronized (Fig. 2*Cc*) nor ventilation related.

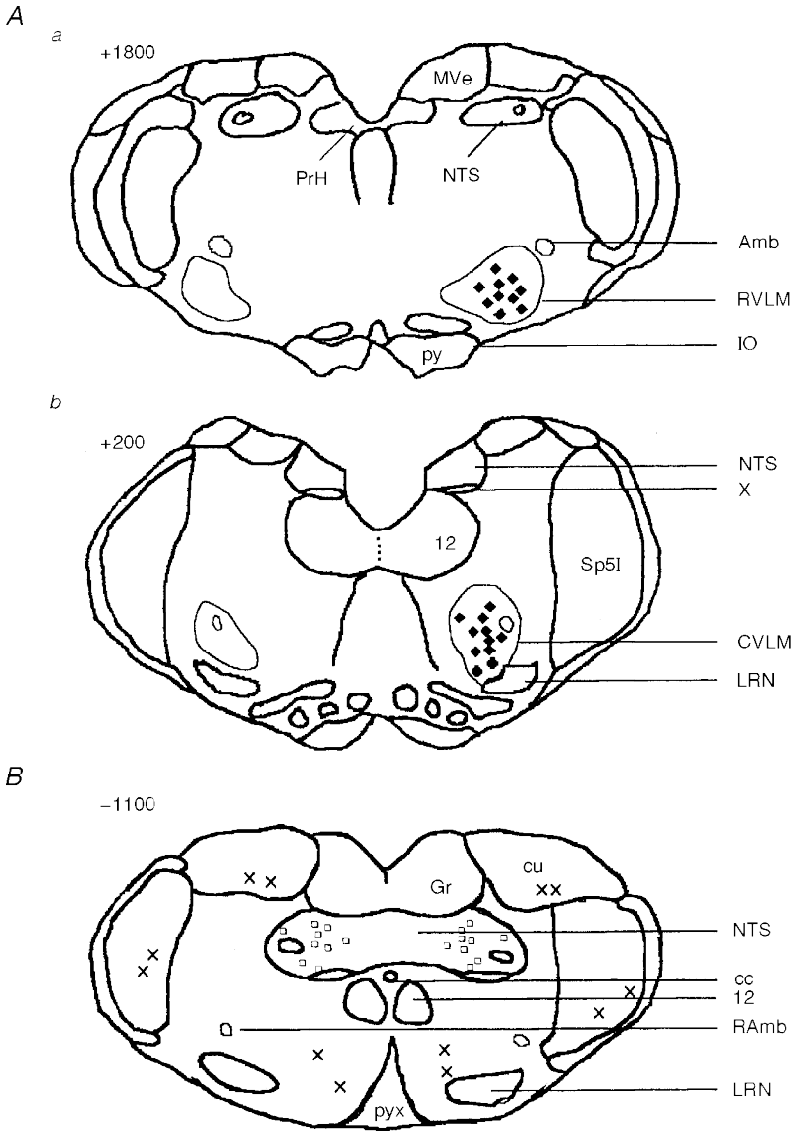

Figure 1. Localization of recording sites in the RVLM and the CVLM, and of microinjection sites of CPBG in different medullary structures

A, camera lucida drawings of frontal sections showing the location of some representative recording sites in the RVLM and the CVLM; *a*, ◆, ten representative cardiovascular (CV-RVLM), respiratory and unidentified RVLM neurones; *b*, ◆, eleven representative CV neurones in the CVLM (CV-CVLM neurones). Sections were cut at ~1800 μm (*a*) and 200 μm (*b*) rostral to the obex. *B*, camera lucida drawing of a frontal section showing the anatomical distribution of CPBG (2 nmol) bilateral microinjection sites. □, nine representative pairs of effective sites; ×, six representative pairs of ineffective sites. In all cases, the symbols point to the centre of microinjection sites. The histological level was ~−1100 μm caudal to the obex. Amb, ambiguus nucleus; CVLM, caudo-ventrolateral medulla; IO, inferior olive; LRN, lateral reticular nucleus; MVe, medial vestibular nucleus; NTS, nucleus tractus solitarii; PrH, prepositus hypoglossal nucleus; py, pyramidal tract; cc: central canal; pyx, pyramidal decussation; RAmb, retroambiguus nucleus; RVLM, rostro-ventrolateral medulla; Sp5I, spinal trigeminal nucleus; X, dorsal vagal motor nucleus; 12, hypoglossal nucleus.

Identification of the CV-CVLM neurones

In agreement with previous data (Jeske, Morrison, Cravo & Reis, 1993), neurones that were excited by a rise in MAP due to inflating the snare or a bolus administration of phenylephrine (5 μg kg^{-1} I.V.) could be identified in the CVLM (eight neurones in eight rats). These neurones were located 1·3–1·5 mm caudal to the CV-RVLM cells, 1·8–1·9 mm lateral to the midline and at a depth of 600–800 μm from the ventral surface of the medulla (Fig. 1*Ab*). The discharge of these CV-CVLM cells, as that of the CV-RVLM neurones, appeared to be pulse synchronized (Fig. 5*A*). Their firing rate was equal to 5·1 ± 2·0 spikes s^{-1} at a baseline MAP of 90·2 ± 3·5 mmHg. After baroreflex activation induced by inflating the snare, their maximal discharge rate reached 10·9 ± 1·0 spikes s^{-1} ($P < 0·05$, Fig. 5*B*).

The effect of Bezold–Jarisch reflex activation on six CV-CVLM neurones was also analysed. Intra-atrial administration of PBG (40 μg kg^{-1}) elicited a marked excitation of these cells (firing rate, 8·2 ± 1·4 spikes s^{-1} from a resting activity of 4·2 ± 2·3 spikes s^{-1}, $P < 0·05$, Fig. 5*B*).

Effects of CPBG microinjections into the NTS

Microinjections of saline into the commissural NTS at the level of the calamus scriptorius (0·3 mm laterally and 0·5 mm in depth) did not produce any significant change in MAP (ΔMAP, −2·1 ± 2·0 mmHg, from a resting MAP of 97·6 ± 1·6 mmHg, $n = 10$). As previously observed in

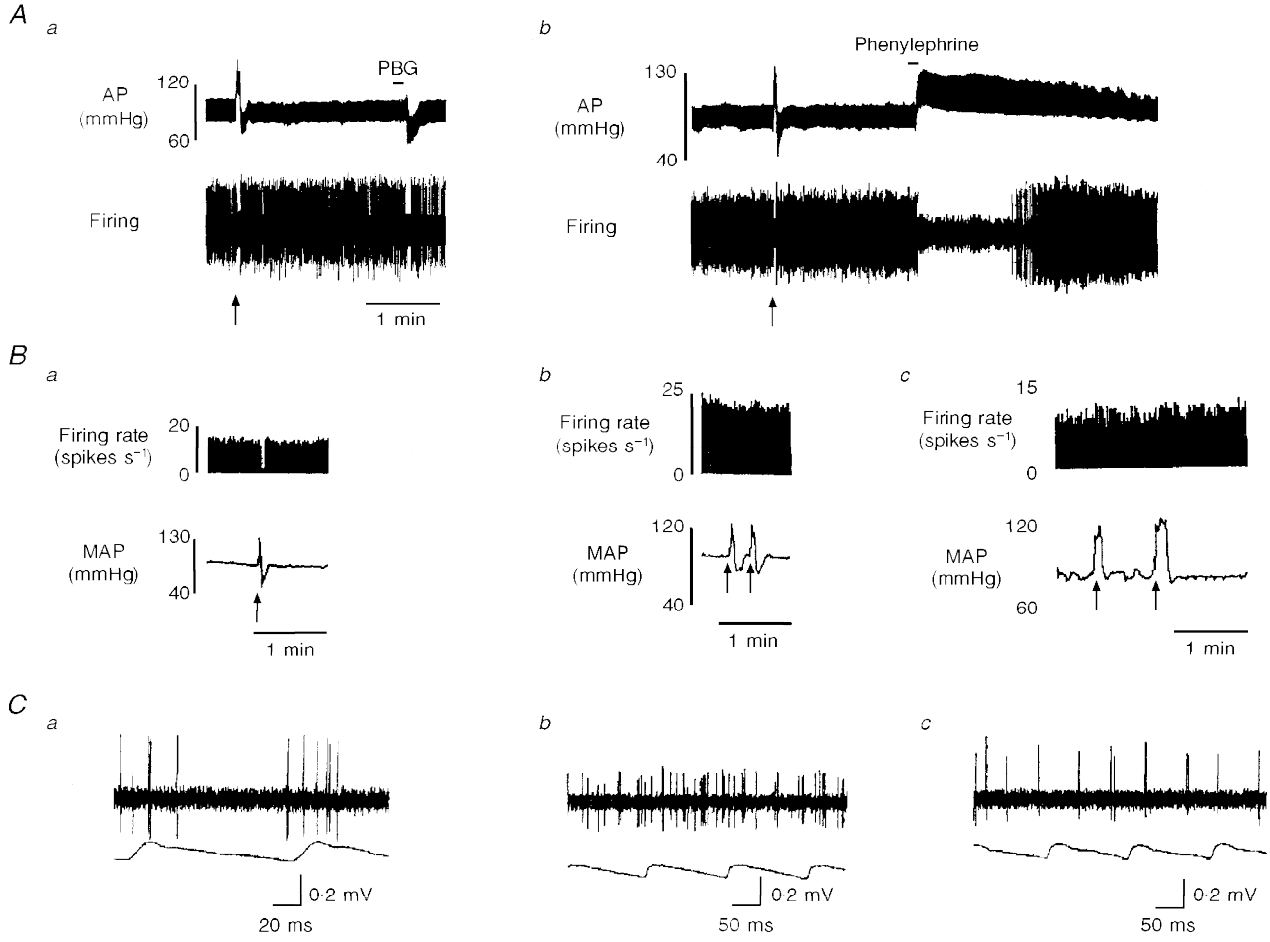

Figure 2. Electrophysiological characteristics of the spinal vasomotor, respiratory and unidentified neurones in the RVLM

A, recording of two CV-RVLM neurones showing the inhibition of firing due to a brief aortic constriction (arrow on the left) (*Aa*) or an I.V. injection of phenylbiguanide (PBG, 40 μg kg^{-1}). Note on the right the hypotension produced by the firing inhibition due to I.V. administration of PBG; *Ab*, a large hypertension elicited by the injection of phenylephrine (5 μg kg^{-1} I.V.). *B*, representative firing rate histograms showing that a CV-RVLM neurone (*a*) was inhibited in response to arterial baroreflex activation, due to brief aortic constriction (arrow). Ventilation-related (*b*) and unidentified (*c*) RVLM neurones were unaffected by aortic constriction (arrows). MAP, mean arterial pressure. *C*, recording illustrating the prominent modulation of the discharge of a typical CV-RVLM (*a*) by the pulse, and the difference of ventilation-related (*b*) and unidentified (*c*) RVLM neurones. Oscilloscopic sweeps were triggered by the pulse. Top, discharges of the neurones (eight sweeps). Bottom, MAP (one sweep).

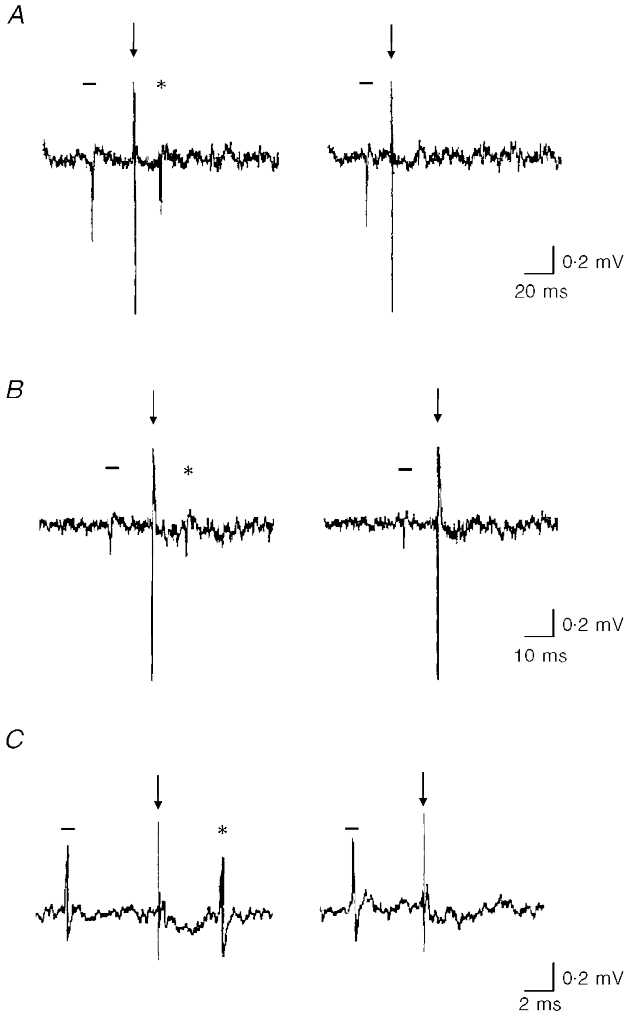

Figure 3. Collision tests identified the CV-RVLM recorded cells as reticulospinal neurones
Stimuli (arrows, 1 Hz, 0·2 mA, 0·2 ms) were delivered through the electrode, with its tip near the intermediolateral column of the spinal cord at the T_2 level (see Methods), after fixed delays following spontaneously occurring spikes (horizontal bar). Evoked spikes (*) were recorded in the RVLM when the stimuli were delivered: *A* (slowest CV neuron) 34 ms (left) but not 20 ms (right), *B* (intermediate CV neuron) 17 ms (left) but not 12 ms (right), *C* (fastest CV neuron) 5 ms (left) but not 4 ms (right), after the spontaneously occurring spikes. Oscilloscopic sweeps were triggered by the spontaneously occurring spikes.

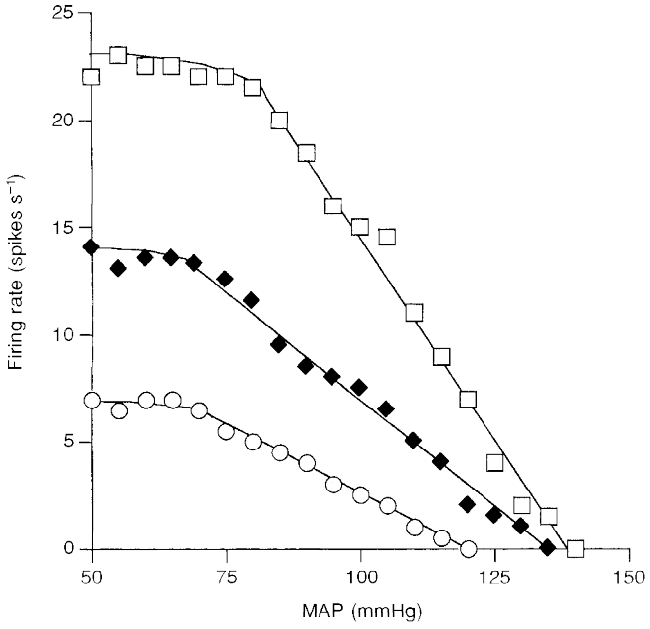

Figure 4. Increase in arterial pressure produced by aortic occlusion inhibited the firing rate of CV-RVLM neurones
Baroreflex activation induced by aortic occlusion with an inflatable cuff produced a progressive and linear decrease in the activity of one representative CV-RVLM neurone of the fastest (□), the intermediate (◆) and the slowest (○) pools (baroreflex curves). The maximum unit activity was measured during the hypotensive period consecutive to the relaxation of the aortic cuff. MAP, mean arterial pressure.

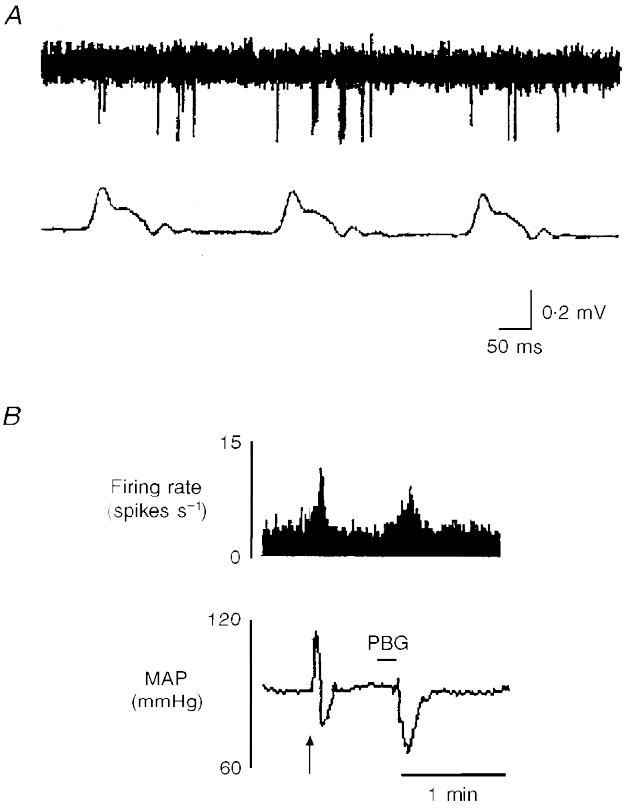

Figure 5. Aortic occlusion and phenylbiguanide administration excited the CV-CVLM neurones

A, recording illustrating the prominent modulation of the discharge of one typical CV-CVLM neurone by the pulse. Top, discharge of the neurone (six sweeps). Bottom, mean arterial pressure (MAP, one sweep). *B*, baroreflex (by aortic occlusion, arrow) and Bezold–Jarisch reflex (by I.V. administration of phenylbiguanide (PBG), 40 μg kg^{-1}) activations increased the firing rate of a CV-CVLM neurone (top). Bottom, increase in MAP by aortic occlusion and hypotensive Bezold–Jarisch reflex response.

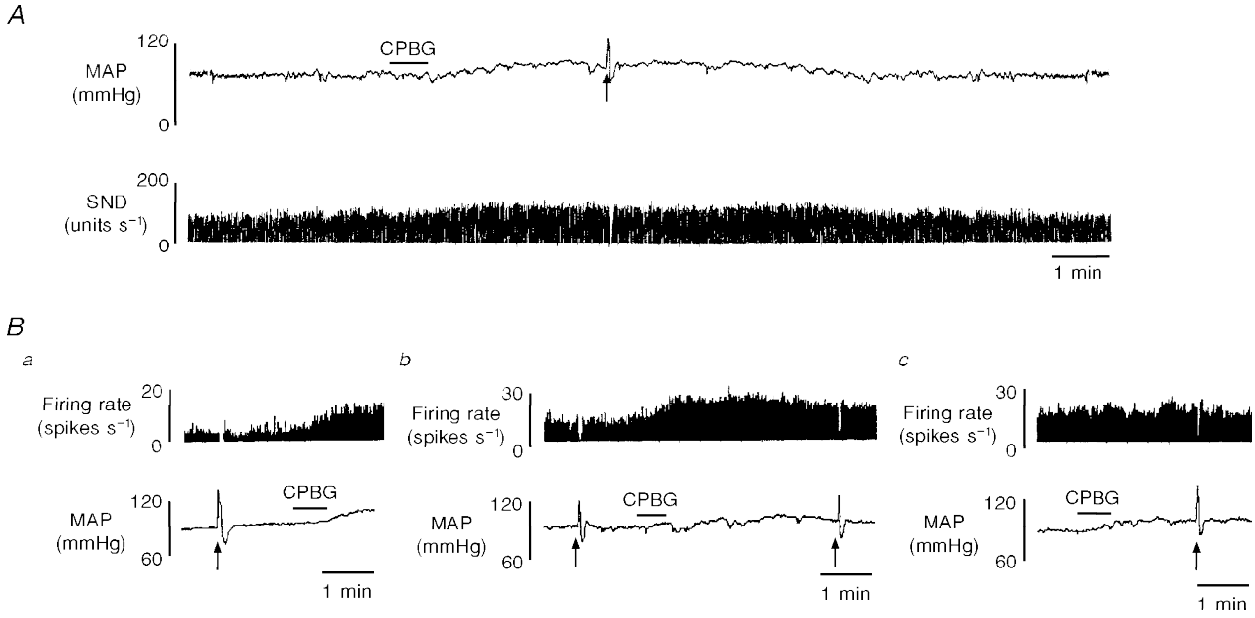

Figure 6. Intra-NTS microinjections of CPBG produced a sympathoexcitation and increased the activity of the slowest and intermediate CV-RVLM neurones

A, bilateral microinjection of the selective 5-HT$_3$ receptor agonist 1-(*m*-chlorophenyl)-biguanide (CPBG, 2 nmol) into the NTS increased the sympathetic lumbar nerve discharge (bottom, SND) and the mean arterial pressure (top, MAP). Arrow, aortic occlusion. *B*, bilateral microinjection of CPBG into the NTS increased the firing rate of a slowest (*a*) and an intermediate (*b*) CV-RVLM neurone, without affecting a fastest (*c*) one. Note the rise in MAP after CPBG microinjections at the bottom. Arrows, aortic occlusion.

urethane-anaesthetized rats (Sévoz *et al.* 1996*b*), 5-HT$_3$ receptor stimulation by bilateral microinjection of a predetermined dose of CPBG (2 nmol) (see Sévoz *et al.* 1996*b*), into the commissural NTS of pentobarbitone-anaesthetized rats, produced a significant increase in both SND (ΔSND, +40·0 ± 5·1 arbitrary units, $n = 10$ rats, $P < 0·05$, see Methods) and MAP (ΔMAP, +22·3 ± 3·3 mmHg, from a resting MAP of 97·3 ± 1·3 mmHg, $n = 10$ rats, $P < 0·05$) (Fig. 6*A*). MAP and SND then returned to baseline values approximately 7 min after the microinjections.

CV-RVLM neurones

Microinjections of saline into the NTS (fifteen rats) did not exert any effect on the basal activity of CV-RVLM neurones (group A, firing rate, 4·7 ± 0·2 spikes s^{-1} from a resting activity of 4·7 ± 0·3 spikes s^{-1}, $n = 5$; group B, firing rate, 9·0 ± 0·2 spikes s^{-1} from a resting activity of 9·2 ± 0·1 spikes s^{-1}, $n = 5$; group C, firing rate, 17·4 ± 0·4 spikes s^{-1} from a resting activity of 17·2 ± 0·3 spikes s^{-1}, $n = 5$). The effects on these cells of CPBG (2 nmol) microinjected into the same sites of the NTS (effective sites of Fig. 1*B*) differed from one cell type to another among the three categories identified in this region of the medulla. Thus intra-NTS CPBG (twenty-three rats) produced an increase in the firing rate of the slowest (group A, firing rate, 8·6 ± 0·3 spikes s^{-1} from a resting activity of 4·8 ± 0·2 spikes s^{-1}, $P < 0·05$, $n = 12$, Fig. 6*Ba*) and intermediate (group B, firing rate, 16·5 ± 0·6 spikes s^{-1} from a resting activity of 9·2 ± 0·4 spikes s^{-1}, $P < 0·05$, $n = 11$, Fig. 6*Bb*) CV-RVLM neurones, which preceded the expected increase in SND and arterial pressure. The discharge rate of these two neuronal populations returned to their basal pre-injection levels approximately 7 min after CPBG microinjections. In addition, intra-NTS CPBG produced a shift to the right of the baroreceptor curves of both A and B neuronal types, which was associated with an increase of the cut-off MAP values (group A, 125·5 ± 2·1 and 155·5 ± 3·1 mmHg, before and after CPBG, respectively; group B, 140·0 ± 2·0 and 190·0 ± 2·5 mmHg before and after CPBG, respectively, $P < 0·05$, Fig. 7*A* and *B*). However, the slopes of these curves were not affected by intra-NTS CPBG (group A, 0·13 ± 0·01 and 0·14 ± 0·01 spikes s^{-1} mmHg^{-1}, before and after CPBG, respectively; group B, 0·20 ± 0·01 and 0·19 ± 0·01 spikes s^{-1} mmHg^{-1}, respectively). In contrast, microinjections of CPBG into the NTS (10 rats) failed to affect the discharge rate of neurones in group C (firing rate, 17·4 ± 0·5 spikes s^{-1} from a resting activity of 17·5 ± 0·4 spikes s^{-1}, $n = 10$) (Fig. 6*Bc*). In addition, the baroreceptor curve of the latter neurones was also unaltered by this treatment. Thus similar slope (0·35 ± 0·01 and 0·37 ± 0·01 spikes s^{-1} mmHg^{-1} before and after CPBG, respectively) and cut-off MAP values (140·2 ± 3·5 and 145·3 ± 3·0 mmHg, before and after CPBG, respectively) were determined for neurones of the C type before and after microinjections of CPBG into the NTS (Fig. 7*C*).

These data show that aortic occlusion-induced inhibition of the CV-RVLM neurones was found to be unchanged after microinjections of CPBG into the NTS (Fig. 6*B*), as it was previously reported for SND after the same treatment

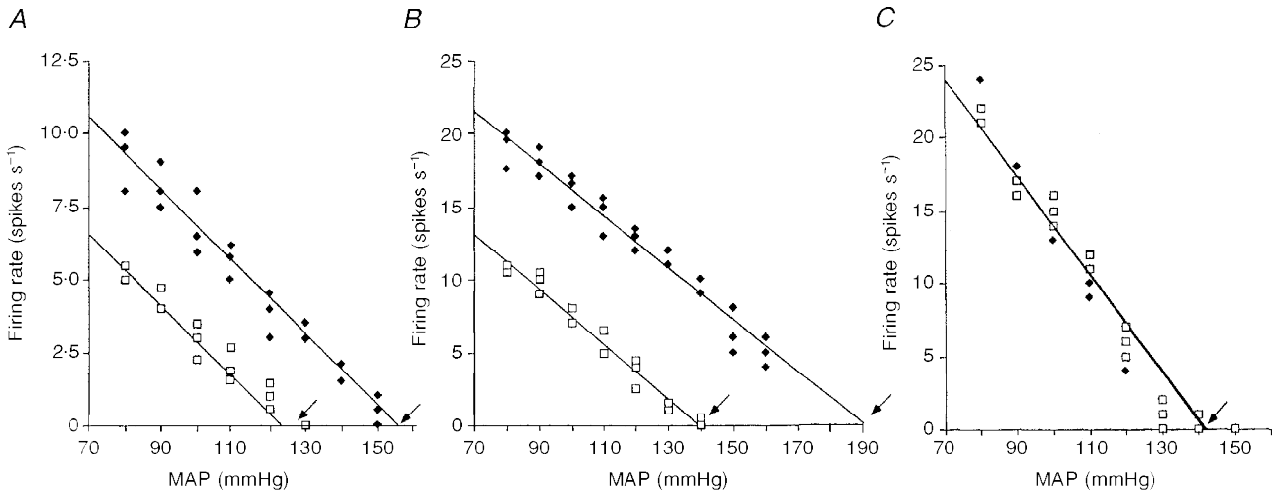

Figure 7. Baroreflex curves of the three cardiovascular pools of CV-RVLM neurones before (□) and after (◆) microinjections of CPBG (2 nmol) into the NTS

Intra-NTS administration of CPBG produced a shift to the right of the baroreceptor curves of the slowest (*A*) and the intermediate (*B*) CV-RVLM neurones, due to an increase in both the activity of these neurones and the cut-off MAP (MAP value above which the neurones were totally silenced, arrows). In contrast, intra-NTS application of CPBG did not modify the baroreceptor curve of the fastest (*C*) CV-RVLM neurones. Note that the slopes of the baroreceptor curves of the three pools of neurones were similar before and after CPBG. Each curve was calculated from data obtained in six different neurones (one neurone per rat).

(Nosjean *et al.* 1995; Sévoz *et al.* 1996*b*). Thus CPBG had no effect on the sympathoinhibition produced by baroreflex activation (sympathetic component).

Finally, in experiments in eighteen rats, we observed that CPBG (2 nmol) microinjected in other medullary structures (Fig. 1*B*) altered neither the MAP baseline nor the firing rate of all the CV-RVLM neurones tested ($n = 18$, one neurone per rat); for instance, in the case of CPBG microinjected into the cuneate nucleus: ΔMAP, $2{\cdot}2 \pm 1{\cdot}5$ mmHg from a resting MAP of $95{\cdot}2 \pm 2{\cdot}1$ mmHg, $n = 6$; group A, firing rate, $4{\cdot}5 \pm 0{\cdot}1$ spikes s^{-1} from a resting activity of $4{\cdot}7 \pm 0{\cdot}4$ spikes s^{-1}, $n = 4$; group B, firing rate, $9{\cdot}2 \pm 0{\cdot}3$ spikes s^{-1} from a resting activity of $9{\cdot}1 \pm 0{\cdot}3$ spikes s^{-1}, $n = 4$; group C, firing rate, $17{\cdot}5 \pm 0{\cdot}4$ spikes s^{-1} from a resting activity of $17{\cdot}3 \pm 0{\cdot}3$ spikes s^{-1}, $n = 4$.

Ventilation-related and unidentified RVLM neurones

Microinjections of saline into the NTS, at the level of the calamus scriptorius, affected neither the basal discharge of the ventilation-related neurones nor that of the unidentified RVLM cells. In addition, intra-NTS microinjections of CPBG (2 nmol) also failed to modify the basal discharge rate of both ventilation-related (firing rate, $20{\cdot}2 \pm 2{\cdot}5$ spikes s^{-1} from a resting activity of $25{\cdot}3 \pm 3{\cdot}5$ spikes s^{-1}, $n = 5$, Fig. 8*A*) and unidentified RVLM neurones (firing rate, $10{\cdot}6 \pm 1{\cdot}0$ spikes s^{-1} from a resting activity of $12{\cdot}7 \pm 1{\cdot}5$ spikes s^{-1}, $n = 5$, Fig. 8*B*).

CV-CVLM neurones

Microinjections of saline into the commissural NTS did not influence the basal activity of CV-CVLM neurones (firing rate, $5{\cdot}7 \pm 0{\cdot}8$ spikes s^{-1} from a resting activity of $5{\cdot}3 \pm 1{\cdot}0$ spikes s^{-1}, $n = 6$, one cell per rat). Similarly, intra-NTS microinjections of CPBG (2 nmol) were inactive on the firing rate of these neurones (firing rate, $5{\cdot}2 \pm 1{\cdot}2$ spikes s^{-1} from a resting activity of $5{\cdot}9 \pm 1{\cdot}5$ spikes s^{-1}, $n = 6$, Fig. 8*C*).

Effects of microinjection of ondansetron into the NTS

As previously reported in urethane-anaesthetized rats (Sévoz *et al.* 1996*b*), bilateral microinjections of a predetermined dose of ondansetron (300 pmol; see Sévoz *et al.* 1996*b*), a specific 5-HT_3 receptor antagonist, at the level of the calamus scriptorius, affected neither the MAP nor the heart

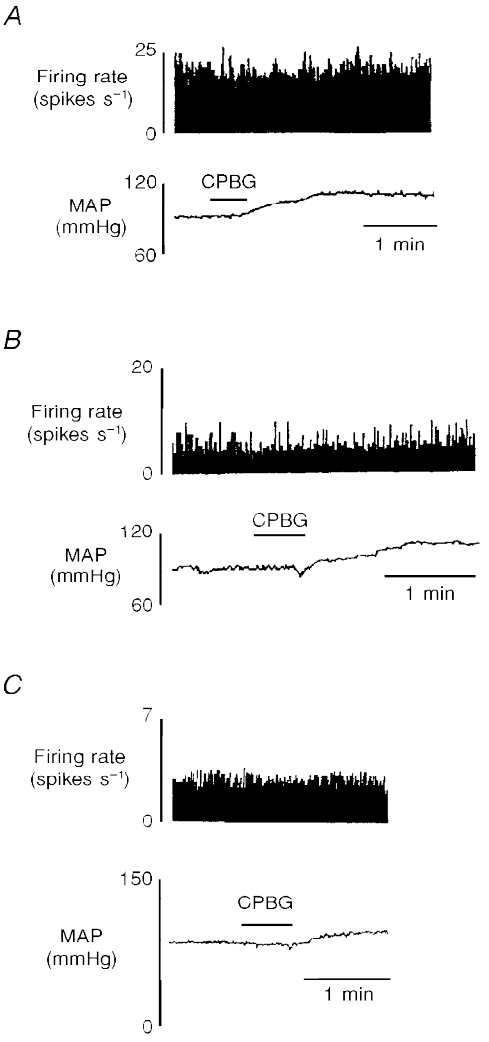

Figure 8. Microinjections of CPBG into the NTS modified neither the activity of ventilation-related and unidentified RVLM neurones nor that of CV-CVLM neurones

Microinjections of CPBG (2 nmol) into the NTS produced no change in the firing rate of a typical ventilation-related RVLM neurone (*A*) and of a typical unidentified RVLM neurone (*B*). Moreover, the same treatment did not affect the firing rate of a typical CV-CVLM neurone (*C*). Note that the mean arterial pressure (MAP) increased following CPBG microinjections.

rate in pentobarbitone-anaesthetized rats ($n = 15$). In addition, no change in the basal activity of CV-RVLM neurones was observed after this treatment (group A, firing rate, $4{\cdot}6 \pm 0{\cdot}1$ spikes s^{-1} from a resting activity of $4{\cdot}8 \pm 0{\cdot}3$ spikes s^{-1}; group B, firing rate, $9{\cdot}0 \pm 0{\cdot}2$ spikes s^{-1} from a resting activity of $9{\cdot}4 \pm 0{\cdot}2$ spikes s^{-1}; group C, firing rate, $17{\cdot}3 \pm 0{\cdot}5$ spikes s^{-1} from a resting activity of $17{\cdot}5 \pm 0{\cdot}3$ spikes s^{-1}, $n = 5$ for each group). However, when injected 2 min prior to CPBG (2 nmol), ondansetron totally blocked the sympathoexcitatory effect of intra-NTS microinjections of CPBG on the activity of the slowest (group A, firing rate, $4{\cdot}6 \pm 0{\cdot}1$ spikes s^{-1} from a resting activity of $4{\cdot}8 \pm 0{\cdot}3$ spikes s^{-1}, $n = 5$) and intermediate (group B, firing rate, $9{\cdot}0 \pm 0{\cdot}3$ spikes s^{-1} from a resting activity of $9{\cdot}4 \pm 0{\cdot}2$ spikes s^{-1}, $n = 5$) CV-RVLM neurones (Fig. 9).

Chemoreflex activation

Stimulation of the carotid chemoreceptors evoked sympathoexcitation and a rise in MAP (Vardhan *et al.* 1993*b*; Koshiya & Guyenet, 1994; Sévoz *et al.* 1997) like that observed after intra-NTS CPBG. In order to compare the effects of these two sympathoexcitatory procedures, we also analysed the effects of the chemoreceptor stimulation on the activity of CV-RVLM neurones.

Chemoreflex activation due to I.V. administration of saline saturated with CO_2 produced a marked increase in both lumbar sympathetic nerve discharge (ΔSND, $+90{\cdot}3 \pm 5{\cdot}5$ arbitrary units, $n = 15$, $P < 0{\cdot}05$, see Methods), and MAP (ΔMAP, $+30{\cdot}2 \pm 5{\cdot}2$ mmHg from a resting MAP of $93{\cdot}5 \pm 5{\cdot}0$ mmHg, $n = 15$, $P < 0{\cdot}05$) (Fig. 10*A*).

However, intracarotid administration of saline saturated with CO_2 failed to affect the rate of the slowest (group A, firing rate, $4{\cdot}5 \pm 0{\cdot}3$ spikes s^{-1} from a resting firing rate of $5{\cdot}0 \pm 0{\cdot}5$ spikes s^{-1}, $n = 5$, Fig. 10*Ba*) and the intermediate (group B, firing rate, $10{\cdot}5 \pm 0{\cdot}5$ spikes s^{-1} from a resting activity of $9{\cdot}3 \pm 0{\cdot}8$ spikes s^{-1}, $n = 5$, Fig. 10*Bb*) CV-RVLM neurones. In contrast, this treatment significantly augmented the discharge rate of the fastest conducting C neurones (firing rate, $30{\cdot}2 \pm 1{\cdot}5$ spikes s^{-1} from a resting activity of $17{\cdot}2 \pm 0{\cdot}5$ spikes s^{-1}, $P < 0{\cdot}05$, $n = 5$, Fig. 10*Bc*). This CO_2-induced increase in the activity of group C neurones was totally prevented by chemodenervation (see Methods, Fig. 10*Bd*).

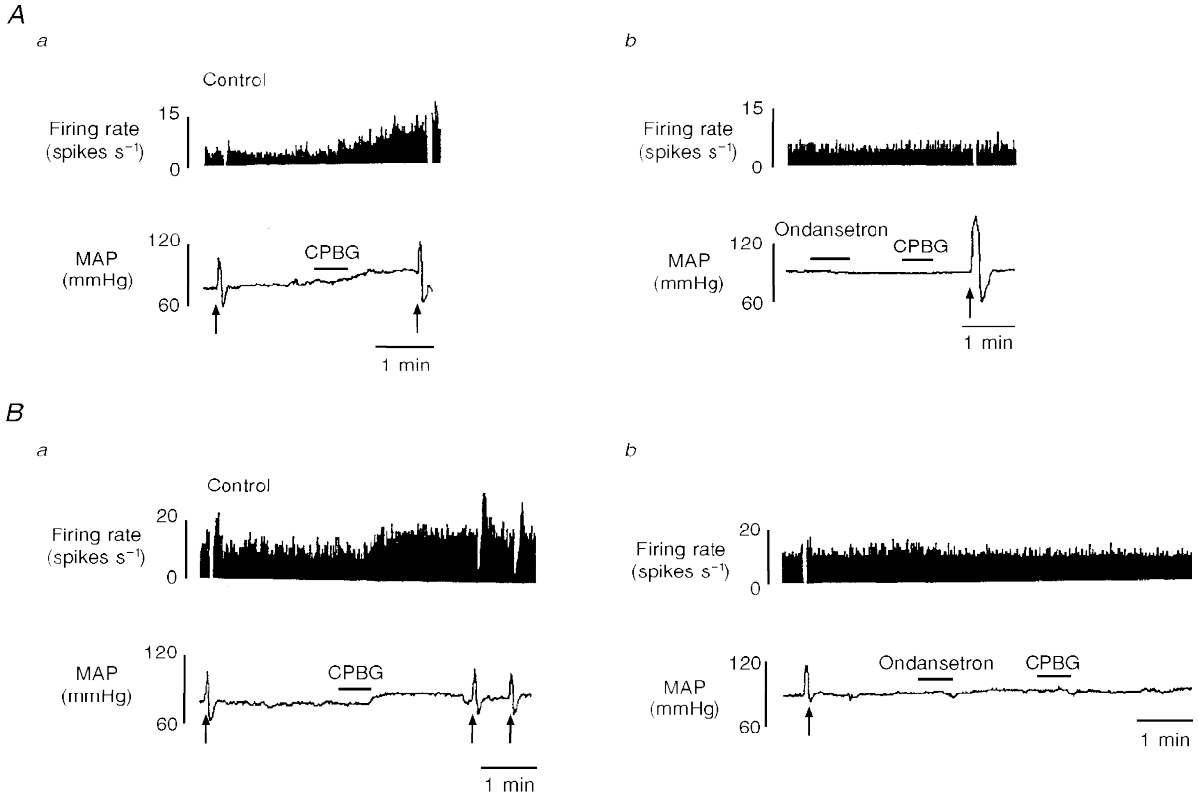

Figure 9. Prior microinjections of ondansetron into the NTS prevented the response of the slowest and intermediate CV-RVLM neurones to local microinjections of CPBG

Control, before bilateral microinjection of ondansetron, a selective 5-HT$_3$ receptor antagonist, microinjections into the NTS of CPBG (2 nmol) excited the slowest (*Aa*) and the intermediate (*Ba*) CV-RVLM neurones, and increased the mean arterial pressure (MAP). After microinjection of ondansetron (300 pmol), the slowest (*Ab*) and the intermediate (*Bb*) CV-RVLM neurones were no longer excited by CPBG (2 nmol), and the CPBG-induced increase in MAP was totally prevented. Arrow, aortic constriction.

DISCUSSION

The present data show that the stimulation, by the potent agonist CPBG, of 5-HT_3 receptors in the region of the commissural NTS led to the excitation of two out of the three different pools of CV-RVLM neurones, without affecting the other neurones in this region. Furthermore, these data confirm that such a stimulation elicits pressor and sympathoexcitatory responses (Nosjean *et al.* 1995; Sévoz *et al.* 1996*b*). As reported previously (Sévoz *et al.* 1996*b*, 1997), the effects of intra-NTS microinjections of nanomolar doses of CPBG are the consequence of the specific activation of NTS 5-HT_3 receptors because they could be blocked by prior local microinjection of ondansetron, a selective 5-HT_3 receptor antagonist. Conversely, prior intra-NTS microinjections of antagonists acting at other 5-HT receptor types did not prevent the effects of CPBG (Sévoz *et al.* 1996*b*, 1997). Altogether, these data support the idea that the stimulation of 5-HT_3 receptors in the NTS increases the arterial pressure and the sympathetic tone through, at least in part, the activation of CV-RVLM neurones.

Identification of CV-RVLM neurones

Previous studies (Brown & Guyenet, 1985; Guyenet & Brown, 1986; Sun & Guyenet, 1986) have clearly shown that RVLM contains different populations of CV neurones, which convey axonal messages to the thoracic spinal cord

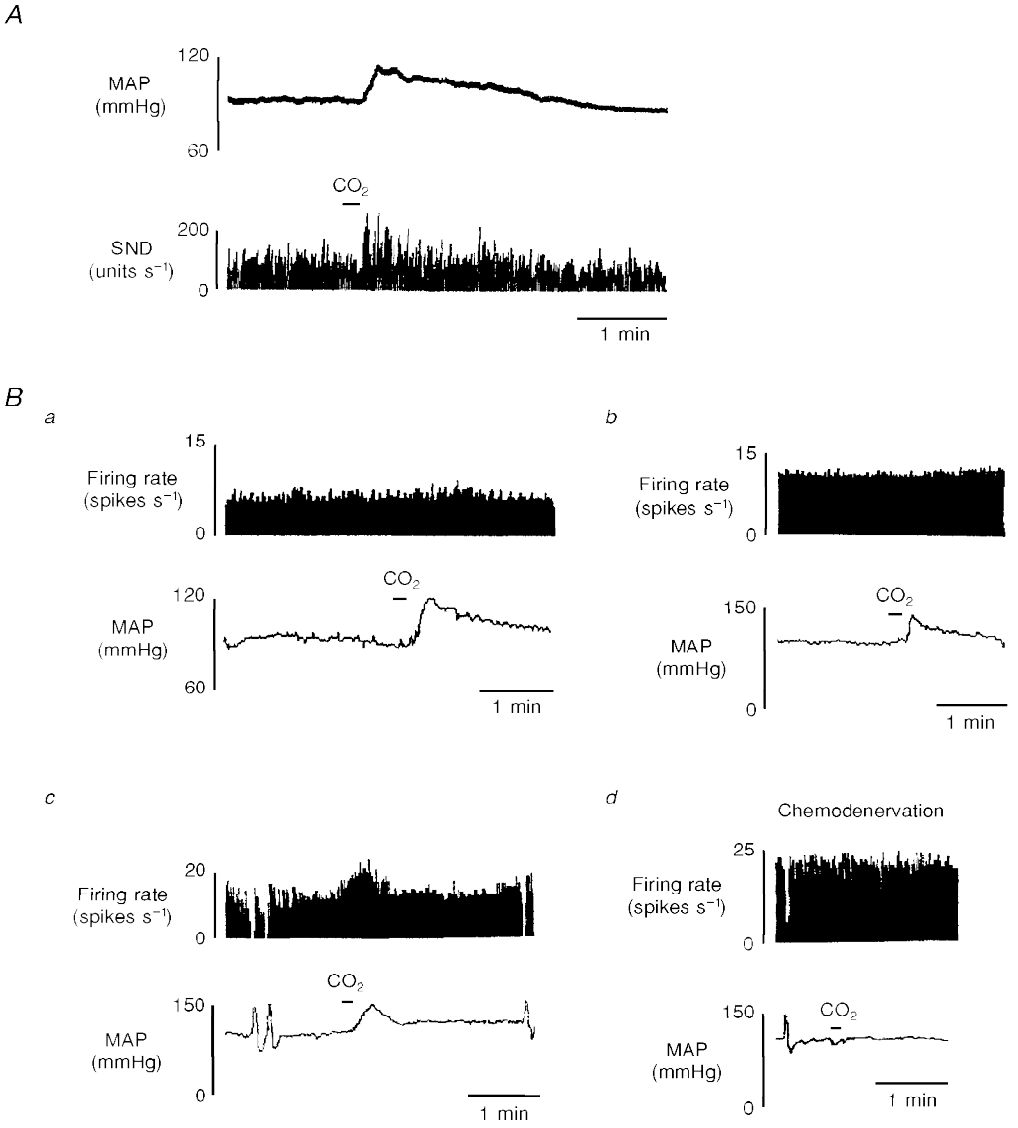

Figure 10. Intracarotid administration of CO_2 produced an increase in the sympathetic nerve discharge and excited the fastest CV-RVLM neurones

A, intracarotid administration of saline saturated with CO_2 elicited an increase in the sympathetic nerve discharge (SND, bottom) and raised the mean arterial pressure (MAP, top). *B*, the same treatment did not affect a slowest (*a*) and an intermediate (*b*) CV-RVLM neurone, but increased the firing rate of a fastest (*c*) one. Panel *Bd* illustrates that the expected CO_2-induced activation of a fastest CV-RVLM neurone and rise in MAP were abolished after chemodenervation. Arrow, aortic constriction.

with different velocities. However, some variations in the characteristics of the different types of CV-RVLM neurones can be pointed out in the relevant literature. These differences are possibly due to the shape of the recording electrode (which may influence types of cells recorded) or the other experimental conditions used. Thus in one of the pioneer studies by Guyenet and his coworkers in halothane-anaesthetized rats (see Sun & Guyenet, 1986), the conduction velocities of the spinal projections of the CV-RVLM cells ranged from 0·4 to 8 m s^{-1}, whereas in urethane-anaesthetized rats, Jeske *et al.* (1993) reported values of 1·1–5 m s^{-1} for the conduction velocity range of the recorded neurones. Under our experimental conditions, in pento-barbitone-anaesthetized rats, the range of axonal conduction velocities of the CV-RVLM neurones was 0·9–7·5 m s^{-1}. In addition, in the study by Sun & Guyenet (1986), two main pools of CV-RVLM neurones were described: the first pool consists of clonidine-sensitive cells with axonal conduction velocities between 0·3 and 0·9 m s^{-1}, and the second one of clonidine-insensitive cells with conduction velocities of 1·5–8 m s^{-1}. However, more recent data obtained in urethane-anaesthetized rats (Koshiya, Huangfu & Guyenet, 1993) suggested the existence of an additional pool of CV-RVLM neurones with an intermediate axonal conduction velocity. Under our conditions in pentobarbitone-anaesthetized rats, we confirmed that CV-RVLM cells are composed of different populations of neurones that can be distinguished by the conduction velocity of their spinal projections, their sensitivity to the intravenous administration of hypotensive doses of clonidine, and also by their averaged discharge rate and their sensitivity to baroreceptor activation. In agreement with Sun & Guyenet (1986), we identified one pool of clonidine-insensitive CV neurones having the highest axonal conduction velocity (ranging between 4·0 and 7·5 m s^{-1}), as well as the highest firing rate and baroreceptor sensitivity. However, in contrast with this previous study (Sun & Guyenet, 1986), we identified two (rather than only one) different pools of clonidine-sensitive CV neurones; the first one is characterized by the slowest axonal conduction velocity (ranging between 0·9 and 1·8 m s^{-1}), firing rate and baroreceptor sensitivity, and the second one has intermediate conduction velocity (from 2·1 to 3·0 m s^{-1}), firing rate and baroreceptor sensitivity.

Specific activation of the clonidine-sensitive CV-RVLM neurones

Our previous results showing that microinjection of kynurenic acid (an antagonist at excitatory amino acid receptors) into the RVLM abolishes the pressor effect of 5-HT$_3$ receptor stimulation in the NTS (Sévoz *et al.* 1996*a*), led us to propose that RVLM neurones are involved in this pressor effect. This hypothesis has been directly assessed in the present study, where we also tried to determine which kind(s) of RVLM neurones transmit the sympathoexcitatory message triggered by the stimulation of 5-HT$_3$ receptors in the NTS. The data reported here clearly show that such a stimulation increases the firing rate of the slowest and the intermediate CV-RVLM neurones (which were both sensitive to clonidine) without affecting the discharge of the fastest CV-RVLM neurones (which were clonidine-insensitive) and of the unidentified (baro-insensitive) and ventilation-related RVLM cells. In addition, previous studies showed that 5-HT$_3$ receptor stimulation in the NTS affected neither the respiratory rate nor the minute ventilation of anaesthetized rats (Sévoz *et al.* 1997). Thus the sympathoexcitatory effect of 5-HT$_3$ receptor stimulation in the NTS appears to be due, at least in part, to the activation of some CV-RVLM neurones, independently of the respiratory network.

CV-CVLM neurones

Since CV-CVLM neurones are not inhibited by 5-HT$_3$ receptor stimulation in the NTS, it can be inferred that these neurones are not involved in the medullary pathway of the resulting sympathoexcitatory message. This is in agreement with recent results showing that (1) microinjection of kynurenic acid into the CVLM, in order to block the baroreflex responses (Jeske *et al.* 1993), did not affect the increase in arterial pressure elicited by intra-NTS CPBG (Sévoz *et al.* 1996*a*), and (2) the latter treatment modified neither the baroreceptor-dependent inhibition of the lumbar sympathetic nerve discharge (Nosjean *et al.* 1995) nor the slope of the baroreceptor curve of CV-RVLM neurones (present data). These data strongly support the idea that the pressor effect produced by the stimulation of 5-HT$_3$ receptors in the NTS is not the consequence of a possible disruption of the tonic baroreceptor inhibition of CV-RVLM neurones. Indeed, whereas baroreceptor inhibition is known to increase the firing rate of all CV-RVLM neurones, intra-NTS CPBG was shown here to increase only the rate of clonidine-sensitive neurones.

Interestingly, the pressor effect elicited by the stimulation of 5-HT$_3$ receptors in the NTS was never associated with a secondary baroreceptor activation of the CV-CVLM neurones. Thus CVLM neurones, which were all excited by the sudden and important increase in arterial pressure elicited by aortic occlusion or phenylephrine administration, were not affected by the slow (2 min were needed for the peak effect) and relatively moderate (around 20 mmHg) increase in arterial pressure produced by CPBG microinjections into the NTS. Further experiments are needed to elucidate the possible physiological relevance of this observation.

Chemoreceptor activation

RVLM is the site of integration of several afferent inputs, originating from peripheral receptors. Thus CV-RVLM neurones also received sympathetic chemoreceptor inputs (Sun & Spyer, 1991; Koshiya *et al.* 1993). Different studies have shown that blockade of excitatory amino acid neuro-transmission in the RVLM prevents both the sympathoexcitation evoked by 5-HT$_3$ receptor stimulation in the NTS (Sévoz *et al.* 1996*a*) and the sympathoexcitatory chemoreflex responses (Sun & Reis, 1995). Moreover, the

latter two sympathoexcitatory responses appear not to be synaptically relayed in the CVLM (Koshiya *et al.* 1993; Sévoz *et al.* 1996*a*; present data). These findings suggest that the pressor response elicited by CPBG administration into the NTS may result from an activation of the sympathetic pathway of the chemoreflex. However, the data reported here show that chemoreflex activation strongly excited the fastest and clonidine-insensitive CV-RVLM neurones (in agreement with Sun & Reis, 1995), whereas 5-HT_3 receptor stimulation in the NTS affected other (e.g. the slowest and intermediate) neurones in this area. Indeed, we found here that intracarotid injection of saline saturated with CO_2 did not affect the latter two categories of CV-RVLM neurones. Our observations on intermediate CV neurones do not agree with those of Koshiya *et al.* (1993), who found that CV-RVLM neurones with corresponding conduction velocities (of $\sim$1·9 m s^{-1}) were excited by chemoreflex activation. However, it has to be emphasized that these authors, although they used experimental conditions different from ours, also reported that a large portion of the recorded CV neurones were not affected by chemoreflex activation (Koshiya *et al.* 1993). Accordingly, the effects of chemoreceptor stimulation on the cell discharge appear to be markedly different, at least for the fastest CV-RVLM neurones, from those evoked by the stimulation of NTS 5-HT_3 receptors. Indeed, we have already shown that microinjection of CPBG into the NTS did not facilitate the sympathetic component of this reflex (Sévoz *et al.* 1997). Taken together, these data suggest that different NTS-RVLM pathways convey the sympathoexcitatory responses of both stimulations.

From all these considerations, it is clear that further experiments are needed to identify the medullary pathway(s) that convey, from the NTS to the RVLM, the messages evoked by 5-HT_3 receptor stimulation. Because 5-HT_3 receptors are known to transmit an excitatory influence of 5-HT on target cells, it can be speculated that the activation of NTS 5-HT_3 receptors located on vagal afferent fibres (Pratt *et al.* 1990) may produce, via the release of a putative excitatory neurotransmitter contained in these fibres (glutamate?), the activation of neurones that project directly or indirectly onto the CV-RVLM neurones. In this respect, it is important to note that monosynaptic projections from the NTS to adrenergic (clonidine-sensitive) neurones in the RVLM have been demonstrated (Hancock, 1988). Thus it can be proposed that a monosynaptic pathway to the RVLM may convey the excitatory message triggered by 5-HT_3 receptor stimulation in the NTS.

Functional considerations

5-HT_3 receptors in the dorso-vagal complex, which includes the NTS, are thought to intervene in vomiting responses in different animal models. However, the NTS is the central structure with the highest density of 5-HT_3 receptors; this is also the case in the rat, which does not vomit (Pratt *et al.* 1990). Furthermore, the present data clearly suggest that the hypothesis concerning the facilitatory role of these receptors in the sympathetic chemoreceptor response can be ruled out. Thus the question of the physiological role(s) of 5-HT_3 receptors in NTS is still pending.

The NTS receives serotoninergic projections from the raphe nuclei (Steinbusch, 1984) and from the nodose ganglia (Nosjean *et al.* 1990). In a previous paper we demonstrated that the serotoninergic terminals originating in the nodose ganglia play a tonic depressor (baroreceptor-like) role in arterial pressure regulation (Orer, Merahi, Nosjean, Fattaccini & Laguzzi, 1991). Since, in contrast, the stimulation of NTS 5-HT_3 receptors produces a clear pressor response, it can be proposed that serotoninergic terminals originating in the raphe nuclei are the source of the 5-HT that stimulates these receptors. In any case, NTS 5-HT_3 receptors do not seem to receive a tonic serotoninergic input because their blockade by local microinjections of ondansetron was found here to exert no influence on the basal level of arterial pressure. Furthermore, Orer *et al.* (1991) previously showed that complete removal of 5-HT by extensive selective lesion of serotoninergic terminals in the NTS induced a transient hypertension, i.e. a change opposite to that expected from the cessation of a hypothetical tonic stimulation of (5-HT_3) receptors mediating a pressor response. Accordingly, it can be postulated that 5-HT_3 receptors may be activated only during circumstances that require a transient cardiovascular adaptation.

It is well established that stimulation of 5-HT_3 receptors in the NTS elicits not only a pressor response, but also a GABA-mediated inhibition of the cardiovagal component of the baroreflex (Merahi *et al.* 1992; Sévoz *et al.* 1996*b*). Sympathoexcitation and cardiovagal inhibition of the baroreflex are parts of the vegetative homeostatic mechanisms that characterize stressful conditions, including fight/flight, defence/attack, somatic and visceral nociception, exercise and mental stress (for review, see Nosaka, 1996). Moreover, the NTS appears to be the gate station of the defence reaction–inhibition of the baroreflex, and some results clearly show that this inhibition is also GABA mediated (Mifflin, Spyer & Withington-Wray, 1988; Jordan, Mifflin & Spyer, 1988). Finally, it has to be emphasized that the raphe nuclei that project to the NTS receive afferents from a great number of central structures involved in stress (i.e. various hypothalamic nuclei, Steinbusch & Nieuwenhuys, 1983). Accordingly, 5-HT_3 receptors in the NTS might be involved in the homeostatic mechanisms that characterize stress and associated behaviours. However, further studies are needed to directly assess this hypothesis.

A clear convergence of sympathetic baroreceptor and cardiopulmonary chemoreceptor (Bezold–Jarisch reflex) messages on some putative CV-RVLM neurones has been clearly demonstrated (Verberne & Guyenet, 1992). The present data also confirm and extend this finding. Thus the three different pools of pulse-synchronized CV-RVLM neurones that project to the spinal cord were inhibited by

both baroreflex and Bezold–Jarisch reflex activation. In addition, both reflexes increase the discharge rate of the CV-CVLM neurones. The present data provide further arguments in favour of the hypothesis that both reflexes share the same integrating mechanisms and pathways in brain (Verberne & Guyenet, 1992; Sévoz *et al.* 1996*b*). In addition, only small and non-significant variations of the discharge rate of the ventilation-related and unidentified RVLM cells were observed upon the activation of the baroreflex or the Bezold–Jarisch reflex. Thus ventilation-related RVLM neurones probably do not play an important role in the integration of the respiratory response (apnoea) produced by both reflexes. Finally, unidentified cells located in the vicinity of the CV-RVLM neurones, which are affected neither by baroreflex (Brown & Guyenet, 1985), Bezold–Jarisch reflex, and chemoreceptor reflex activation, nor by 5-HT$_3$ receptor stimulation in the NTS (present data), are apparently not involved in the reflex regulation of the sympathetic tone.

In summary, the present study showed that the sympathoexcitation and the increase in arterial pressure elicited by the stimulation of 5-HT$_3$ receptors in the NTS result from the activation of two different pools of clonidine-sensitive CV neurones of the retrofacial region of the RVLM. The present data also demonstrated that all the CV-RVLM neurones integrate the inputs from baroreceptors and cardiopulmonary (Bezold–Jarisch reflex) receptors, and confirmed that the 5-HT$_3$ receptor-evoked sympathoexcitation is not the consequence of some inhibition of baroreceptor inputs. In addition, our data showed that the CV-RVLM neurones excited by chemoreceptor inputs are not those that are activated by 5-HT$_3$ receptor stimulation in the NTS. Thus the 5-HT$_3$ receptor-evoked sympathoexcitation does not share the same RVLM integrating mechanisms as those of the sympathetic component of the chemoreflex. These data are compatible with a physiological role of NTS 5-HT$_3$ receptors in a phasic control of arterial pressure, notably during stress and associated behaviours.

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